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1996 Jan, 2(1):52-58

3. Advances in Experimental Medicine and Biology, 2001, Vol. 495 (progress in basic and clinical immunology), pp. 349-354.

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Exosomes in cancer immunotherapy: preclinical data

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1. INTRODUCTION

1.1 Dendritic cells in cancer immunotherapy : results of pioneering clinical trials

Tumor cells present tumor antigens, but they are not immunogenic. To circumvent this lack of immunogenicity and induce expansion of specific cytotoxic T lymphocytes (CTL), some investigators have proposed to use dendritic cells in cancer immunotherapy. Dendritic cells (DC) are unique professional antigen presenting cells that are able to uptake and cross-present class I and class II restricted antigens to CD8+ and CD4+ T lymphocytes. Importantly, mature DC are also capable of priming naive T lymphocytes through expression of costimulatories molecules (CD80, CD86). Proof of principle of the role of dendritic cells in clinical trials has been reported in normal volunteers by Dhodapkar (1). Indeed, DC pulsed with antigens, but not DC alone, nor peptides alone, were able to induce primary and to boost secondary antigen specific T cell based immune response. Some clinical trials using this strategy for metastatic cancer treatment have been reported. Using ex-vivo propagated DC, pulsed with either peptides or tumor cell lysates, Nestlé et al (2) reported 5 clinical responses in 16 melanoma

Progress in Basic and Clinical Immunology, Edited by Mackiewicz *et al.*
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patients. Using fusion between DC and tumor cells, Kugler et al (3) reported 4 complete responses in 17 metastatic renal cancer patients.

1.2 Dendritic cells in cancer immunotherapy: prospect and limitations

Monocytes derived and CD34 derived DC pulsed with peptides or lysates or apoptotic bodies are currently tested in several clinical trials. Preliminary data ascertain their lack of toxicity. DC appear to elicit Ag specific antitumor immune response in patients leading to some objective tumor regressions. They are currently tested in phase III clinical trial in patients with prostate cancer and melanoma.

Although it is a promising therapy for cancer patients, some shortcoming need to be mentionned. Two major points are of importance. As any autologous cell therapy, there is a phenotypic variability as a function of patient clinical condition. This represents a hindrance for exploitation and dissemination plans in term of upscale and manufactoring. The option of replacing DC by a component that exhibit immunostimulatory properties of DC but pharmaceutical properties of a drug (stable phenotype, well defined composition, dosage, manufacturing process, allogenic use) is particularly interesting. The second important point to be considered in DC therapy is the method for pulsing. Pulsing must be performed with components that allow a polyepitopic CTL expansion to avoid tumor immunoselection. In addition, the Ag delivery system for optimal DC loading must take into account the optimal pathway of antigen cross-presentation. To date, only apoptotic bodies (4) and immune complexes (5) have been shown to induce antigen cross-presentation. Finally, the method used for DC pulsing must be well characterized for optimal therapeutic use.

1.3 Exosomes: a cell derivative for biotechnology

Exosomes have been described as small membrane vesicles of 60-90 nm released by reticulocytes (6). Later on, Raposo et al (7) found that B-lymphocytes were also able to release exosomes. Molecular characterisation showed that reticulocytes-derived exosomes contain Transferin-receptor, $\alpha 4\beta 1$ integrins, Heat Shock Protein (HSP) and that B Lymphocytes derived exosomes exhibit functional MHC class II/peptides complexes on their surface.

Therefore, exosomes originate from late endosome and are released by a broad array of hematopoietic cells. They contain protein involved in functions of the cells from which they derive. Considering these data and the

immunostimulatories properties of DC, we investigated the ability of DC to secrete immunostimulatory exosomes.

2. DENDRITIC CELL-DERIVED EXOSOMES: A POTENTIAL CELL-FREE ALTERNATIVE TO DC THERAPY

2.1 Tumor peptide-pulsed DC secrete exosomes that induce tumor growth suppression in mice (8)

Immunoelectronic microscopy studies have shown that murine dendritic cells secrete exosomes (Dex) that exhibit high amounts of MHC class I and II molecules and tetraspanin (CD63). Therefore, we tested the immunogenicity of such exosomes and their potential antitumor effet in mice. BM-DCs were pulsed with acid-eluted tumor peptides from P815 and DC supernantant were harvested to purify Dex. After a single intradermal injection of these Dex, 40 to 60% of P815 bearing mice were free of tumor at day 60. In addition, these animals rejected lethal injection of the syngeneic P815, while no rejection occured after injection of irrelevant L1210 tumor cells. These findings suggest that Dex were able to induce a long lasting antitumor immune response. No effect of Dex were found in Athymic Nu/Nu mice, indicating that T cells are required for the exosome-induced antitumor immune response *in vivo*.

2.2 Molecular composition of murine Dex: enrichment in proteins involved in the immunological network (8,9)

To unravel the molecular basis of the immune effects of Dex, a molecular characterization has been performed. Dex exhibit large amounts of MHC class I and II molecules and tetraspanins (CD63, CD81, CD82, CD9) on their surface. Analysis of protein profile of the Dex identified eight more dominant proteins. Three are transmembrane (Mac-1α chain, MHC class IIB chain and CD9), one is associated to membranes (MFG-E8), and four are cytosolic (Gi2α, annexin II, gag from MRV provirus, hsc73). Two of these components seem especially relevant for Dex functional activity. The first is hsc73, expressed constitutively and reported to be immunogenic (10). The other interesting component is MFG-E8. This protein has been initially

described at the surface of milk fat globule. This is a protein involved in cell-cell contact and interacts through αVβ3 and αVβ5 integrins (11).

2.3 Human MD-DC secrete exosomes that bear functional MHC class I/peptide complexes (12)

Electronic microscopy and western-blot analysis showed that human ex-vivo propagated MD-DC secrete exosomes. These exosomes contain MHC class I and II molecules. In vitro data suggest that human Dex are antigenic on CTL clones and therefore bear functional MHC class I and class II/ peptides complexes on their surface.

2.4 Conclusion and perspectives

Exosomes are 60-90 nm membranes vesicles originating from late endosomes and constitutively secreted by DC. They mediate T cell-based immunogenicity *in vitro* and in tumor bearing mice. Dex represents an alternate immunotherapy strategy to DC in *in vivo* preclinical models. Exosomes are stable and well defined and could be manufactured in biotechnology. A clinical grade upscale production of Dex and quality control parameters have been recently established. This translational research allowed implementation of such a cancer vaccine strategy into the clinic. A phase I clinical trial in metastatic melanoma and lung cancer has been recently launched. Research aimed at identifying the critical molecules involved in Dex biogenesis and function is underway and may allow de novo neosynthesis of bioactive exosomes.

3. TUMOR CELL-DERIVED EXOSOMES (TEX): A NEW ANTIGEN DELIVERY SYSTEM FOR DC LOADING (13)

3.1 Results

Tumor cell lines in mouse and human systems release HSP and Class I containing exosomes. Interestingly, western blot analysis revealed that Tex also vehicle tumor antigens such as Mart1, tyrosinase related proteins (TRP). Tex from a HLA-A2$^-$ Mart1+ tumor cell line induce cross-presentation of Mart1 tumor antigen to a CTL clone. In addition, Tex pulsed onto DC protect mice against tumor challenge. Exosomes were immunogenic across

histology since MC38 derived exosomes (colon cancer) were efficient to protect mice against TS/A challenge (breast cancer).

3.2 Perspectives

Tex are constitutively released by tumor cells, and are a source of tumor rejection antigens for CTL cross-priming. They are immunogenic across tumor histology and MHC class I barriers. Compared with other methods of polyepitopic DC pulsing (apoptotic bodies, tumor lysates, mRNA...), they exhibit properties that render them attractive. They are stable, their composition can be well defined, and their storage and safety profile might comply with regulatory agencies requirements. Apart for being an attractive method for DC pulsing, Tex are also a novel source of tumor rejection antigens. Cloning of CTL induced by DC pulsed with Tex should allow identification of new tumor epitopes.

REFERENCES

1. Dhodapkar MV, Steinman RM, Sapp M, Desai H, Fossella C, Krasovsky J, Donahoe SM, Dunbar PR, Cerundolo V, Nixon DF, Bhardwaj N, 2000, Rapid generation of broad T-cell immunity in humans after a single injection of mature dendritic cells.*J Clin Invest* 10: 19-14
2. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D, 1998, Vaccination of melanoma patients with peptides- or tumor lysate-pulsed dendritic cells. *Nat Med* 4 : 328-32
3. Kugler A, Stuhler G, Walden P, Zoller G, Zobywalski A, Brossart P, Trefzer U, Ullrich S, Muller CA, Becker V, Gross AJ, Hemmerlein B, Kanz L, Muller GA, Ringert RH, 2000, Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids. *Nat Med* 6 : 332-6
4. Albert, M. L., Sauter, B. and Bhardwaj, N., 1998, Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs, *Nature* 392. 86-89
5. Regnault, A., Lankar, D., Lacabanne, V., Rodriguez, A., Thery, C., Rescigno, M., Saito, T., Verbeek, S., Bonnerot, C., Ricciardi-Castagnoli, P. and Amigorena, S., 1999, Fc gamma receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization, *J. Exp. Med.* 189. 371-380
6. Johnstone, R. M., Adam, M., Hammond, J. R., Orr, L. and Turbide, C., 1987, Vesicle formation during reticulocyte maturation, *J. Biol. Chem.* 262. 9412-9420
7. Raposo, G., Nijman, H. W., Stoorvogel, W., Leijendekker, R., Harding, C. V., Melief, C. J. M. and Geuze, H. J., 1996, B lymphocytes secrete antigen-presenting vesicles, *J. Exp. Med.* 183. 1161-1172
8. Zitvogel, L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G, Amigorena S, 1998, Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat. Med.* 4, 594-600

9. Thery, C., Regnault, A., Garin, J., Wolfers, J., Zitvogel, L., Ricciardi-Castagnoli, P., Raposo, G. and Amigorena, S., 1999, Molecular characterization of dendritic cell-derived exosomes: selective accumulation of the heat shock protein hsc73, *J. Cell. Biol.* 147. 599-610

10. Srivastava, P. K., Menoret, A., Basu, S., Binder, R. J. and McQuade, K. L., 1998, Heat shock proteins come of age: primitive functions acquire new roles in an adaptive world, *Immunity.* 8. 657-665

11. Andersen MH, Graversen H, Fedosov SN, Petersen TE, Rasmussen JT, 2000, Functional analysis of two cellular binding domains of bovine lactadherin. *Biochemistry* 39 : 6200-6

12. Wolfers J, Andre F, Ruegg C, D. Shu, V. Serra, O. Dhellin, G. Raposo, C. Thery, S.Amigorena, J. Crouzet, T.Tursz and L.Zitvogel, 2000, Dendritic cell-derived exosomes are effective cancer vaccine : preclinical data towards a clinical trial. *Proc Am Ass Cancer Res*, A5556

13. Wolfers J, Lozier A, Raposo G, Regnault A, Thery C, Masurier C, Flament C, Pouzieux S, Faure F, Tursz T, Angevin E, Amigorena S, Zitvogel L, 2001, Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. *Nat Med*, in press